

REMARKS

In the foregoing Listing of Claims, Applicants cancel claim 1 and amend claims 18 and 22 by further defining a method of inhibiting tyrosinase activity in a subject in need of preventing or alleviating spots and freckles created on skin or a method of ameliorating facial blood flow in a subject in need thereof, which comprises administering a composition comprising an effective amount of anthocyan for suppressing preventing or alleviating spots and freckles created on skin or for ameliorating facial blood flow to the subject. These aspects of Applicants' invention are described on page 5, lines 6-18; page 8, lines 12-17; and elsewhere in the Specification. Applicants respectfully request reconsideration and allowance of the inventions defined in claims 18-25 for reasons that follow.

Applicants desire to express thanks to Examiner Elli Peshev for the courtesies extended the undersigned in a telephone interview on January 22, 2010. During the interview, the foregoing amendments to claims 18 and 22 were discussed among other things including the alleged inherency of the presently claimed method within the teachings of Matsumoto (EP 1 208 755 A1). Examiner Peshev stated that amended claim 22 has a very good chance of patentability. With respect to amended claim 18, Examiner Peshev stated this claim has a good chance of patentability, but she would have to consider this matter further after a response is filed.

The Office Action included a single prior art rejection of claims 1 and 18-25 under 35 U.S.C. §102(b) as being anticipated by Matsumoto. Matsumoto was used to reject Applicants' claims in previous Office Actions. The Office Action took the position that Applicants' claimed tyrosinase inhibiting activity and amelioration of facial blood flow activity would have been inherent in the method disclosed by Matsumoto. In the foregoing amendments, Applicants

cancel claim 1. In addition, Applicants amend claim 18 by defining a method of inhibiting tyrosinase activity in a subject in need of preventing or alleviating spots and freckles created on skin, which comprises administering a composition comprising an effective amount of anthocyan for preventing or alleviating spots and freckles created on skin to the subject. Similarly, Applicants amend claim 22 by defining a method of ameliorating facial blood flow in a subject in need thereof, which comprises administering a composition comprising an effective amount of anthocyan for ameliorating facial blood flow to the subject. Applicants respectfully submit that the presently claimed method of inhibiting tyrosinase activity in a subject in need of preventing or alleviating spots and freckles created on skin and the presently claimed method of ameliorating facial blood flow in a subject in need thereof, which comprises administering a composition comprising an effective amount of anthocyan for preventing or alleviating spots and freckles created on skin or for ameliorating facial blood flow to the subject, are not and cannot be inherent within the teachings of Matsumoto. Therefore, the presently claimed methods as defined in claims 18-25 cannot be anticipated by Matsumoto within the meaning of 35 U.S.C. §102.

Applicants respectfully submit that the presently claimed use of inhibiting tyrosinase activity in a subject in need of preventing or alleviating spots and freckles created on skin as required in present claims 18-21 is not related to the uses of improving visual function, improving body fluidity, and/or lowering blood pressure as discussed in Matsumoto, and thus is a new use over Matsumoto.

Applicants speculate that the mechanism of inhibiting tyrosinase is as follows. When epidemic cells are irradiated with ultraviolet light, a signaling substance that enhances the synthesis of melanine is produced. The signaling substance binds to melanocytes. The

melanocytes are then activated and grow, and produce and activate tyrosinase that is a melanine-producing enzyme. The activated tyrosinase converts tyrosine to DOPA (dihydroxyphenylalanine) in a living body and then dopaquinone which stimulates the production of melanine is produced. Accordingly, inhibiting tyrosinase inhibits the production of DOPA and dopaquinone, which in turn inhibit the production of melanine. The attached Exhibit A (K. Ohhara et al., Functional Food, 2009, Vol. 2, No. 4, p. 383-386) shows the mechanism, especially in Fig. 3.

The teachings of Matsumoto never disclose nor suggest the tyrosinase-inhibiting activity of anthocyanin and any relationship between tyrosinase-inhibiting activity and the production of melanine in a subject in need of preventing or alleviating spots and freckles created on skin as required in present claims 18 to 21. In addition, these properties or functions of the inventions defined in claims 18 to 21 are not related to the uses of improving visual function, improving body fluidity, and/or lowering blood pressure as proposed by Matsumoto, and thus is a new use or utility over Matsumoto. At least for these reasons, Applicants respectfully submit that the inventions of claims 18 to 21 are not anticipated by Matsumoto, and thus, are patentable thereover.

Applicants' claims 22 to 25 are directed to a method of ameliorating facial blood flow in a subject in need thereof, which comprises administering a composition comprising an effective amount of anthocyan for ameliorating facial blood flow to the subject. This claimed use is not related to the uses of improving visual function, improving body fluidity, and/or lowering blood pressure as discussed in Matsumoto, and thus is a new use over Matsumoto.

At best, Matsumoto proposes that an anthocyanin-containing composition has a blood fluidity improvement function. However, the blood fluidity improvement function discussed in

Matsumoto is different from the function for improving facial blood flow that is required in the present claims. In particular, Matsumoto describes the blood fluidity effects in Example 12. Example 12 discloses “This fresh whole blood obtained by the collection of heparin was poured into a micro channel array (width 7 μm , height 30 μm , depth 4.5 μm , and 8736 channels (Bloody 6-7, Hitachi Haramachi Electronics Co., Ltd.) at a water column difference of 20cm using MC-FAN (Santuri Kiko). The time necessary for 100 μl to pass through was determined.” That is, the “improving blood fluidity function” is evaluated by collecting blood measuring the time for blood to pass through the micro channel array. Attached Exhibit B is a copy of the catalogue of the MC-FAN used in the experiment of Example 12. Pages 2 and 4 of Exhibit B show the micro channel array. The width of the micro channel array is 7 μm , which is the same as the diameter of a blood capillary. Page 2 of Exhibit B includes examples that describe how the analyzer is used. It is clear that the fluidity of blood component such as erythrocytes, leukocytes, and platelets was measured in Example 12 of Matsumoto. That is, the alleged “blood fluidity improvement function” of Matsumoto is a function to improve the fluidity of blood components such as erythrocytes, leukocytes, and platelets. Furthermore, Matsumoto describes, “That is, according to the present invention, diseases such as cerebral by affecting erythrocytes, leukocytes, and platelets as such in the blood to improve the fluidity of the blood itself, thereby lowering blood pressure rather than by vasoconstriction” in paragraph [0095] of EP 1208755 A1.

On the contrary, the blood flow improving function of the presently claimed inventions is based on vasodilatation effect on peripheral vessels. This function is significantly different from and unrelated to the blood fluidity improvement function discussed in Matsumoto.

The attached Exhibit C (Iwasaki-Kurashige et al., Vascular Pharmacology 44 (2006) 215-223) shows that blackcurrant concentrate which includes anthocyanin decreases peripheral vascular resistance that results in vasodilatation. Exhibit C illustrates the blood flow improving function required in claims 22-25. Furthermore, the presently claimed method has the advantageous effect of ameliorating facial blood flow within 15 minutes.

Accordingly, the claimed inventions in claims 18 to 21 are based on the newly found function of anthocyanin for improving facial blood flow, which function is not describe nor inherent in Matsumoto. It is well established in the case law that the discovery of a new use for an old structure based on unknown properties of the structure might be patentable to the discoverer as a process of using. *In re Hack*, 245 F.2d 246, 248, 114 USPQ 161, 163 (CCPA 1957). In US patent practice, many patents have been issued for novel use of known substances. For example, minoxydil had been patented as a blood pressure-lowering drug (US 3,461,461). Then, the new effect of minoxydil for enhancing hair growth was discovered and minoxydil was patented as a hair growth stimulant (US 4,139,619). Applicants respectfully submit that the methods defined in claims 18-25 fall within this category of invention. Namely, the presently claimed method of inhibiting tyrosinase activity in a subject in need of preventing or alleviating spots and freckles created on skin and the presently claimed method of ameliorating facial blood flow in a subject in need thereof, which comprise administering a composition comprising an effective amount of anthocyan for preventing or alleviating spots and freckles created on skin or for ameliorating facial blood flow to the subject, are new uses for the compounds set forth in the present claims, which are not and cannot be inherent within the teachings of Matsumoto. At least for this reason, the inventions defined in claims 18-25, which are based on new uses of anthocyanin, are patentable.

At least for the foregoing reasons, Applicants respectfully submit that the presently claimed invention is patently distinguishable from Matsumoto. Therefore, Applicants respectfully request that the Examiner reconsider and withdraw any §102 or §103 rejection of method claims 18-25 over the teachings of Matsumoto.

Applicants believe that the foregoing is a complete and proper response to the Office Action mailed September 23, 2009. While it is believed that all pending claims in this application are in condition for allowance, if the Examiner has any comments or questions, Applicants invite the Examiner to telephone the undersigned to resolve any outstanding issues at the below listed number.

In the event this paper is not timely filed, Applicants hereby petition for an appropriate extension of time. The Commissioner is hereby authorized to charge the fee therefor, as well as any other fees which become due, to our Deposit Account No. 50-1147.

Respectfully submitted,

/R. Eugene Varndell, Jr/
R. Eugene Varndell, Jr.
Attorney for Applicants
Registration No. 29,728

Posz Law Group, PLC
12040 South Lakes Drive, Suite 101
Reston, VA 20191
Phone 703-707-9110
Customer No. 23400

ATTACHMENTS:

EXHIBIT A ~ K. Ohhara et al., Functional Food, 2009, Vol. 2, No. 4, p. 383-386 (5 pp.).

EXHIBIT B ~ Catalogue of MC-FAN (4 pp).

EXHIBIT C ~ Iwasaki-Kurashige et al., Vascular Pharmacology 44 (2006) 215-2236 (6 pp.).

Exhibit A

Partial English translation of K. Ohhara et al., Functional Food, 2009, Vol.2, No.4, p.383-386

Partial translation of K. Ohhara et al., Functional Food, 2009, Vol.2, No.4, p.383-386

Special topic Aging of skin and functional food

6. Effects of functional components in food for improving and preventing spots and cockles

Hiroki Ohhara¹⁾, Shingo TAJIMA²⁾

1) Meiji Seika Kaisha, Ltd, Food and Healthcare Research Institute

2) National Defense Medical College Hospital

Abstract

lines 6 to 8

It has been suggested that cassia-anthocyanin components which are transferred in blood inhibits tyrosinase activity which involves in producing melanine and ameliorating impaired blood circulation and takes effect on spots.

Figure 3 Hypothesis for effects of cassia-anthocyanin on improving and preventing spots

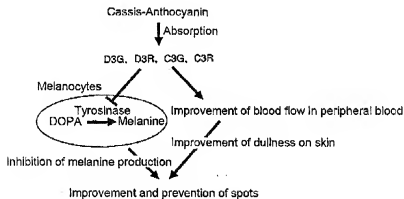


Exhibit A
page 1
(English)

Exhibit A

特集 皮膚老化と機能性食品

6. 食品機能成分のシミ、シワの改善と 予防効果

大原 浩樹¹⁾ 多島 新吾²⁾¹⁾ 明治製菓株式会社食料健康総合研究所²⁾ 防衛医科大学校皮膚科学講座

皮膚の老化に伴って発生するシミやシワを改善・予防することはQOL (quality of life) 向上にも重要である。近年、予防的な観点から食品機能成分の皮膚に対する効果について研究が進められ、その生理機能が明らかになってきている。そこで、我々はカシスアントシアニンとコラーゲンペプチドについて研究を進め、シミとシワの改善・予防効果を見出した。

カシスアントシアニンは、血中に移行したカシスアントシアニン成分がメラニン生成に関与するチロシナーゼ活性を阻害し、さらに血流不全を改善することでシミに対して効果があることが示唆された。一方、コラーゲンペプチドは、血中に移行したHyp (ヒドロキシプロリン) 含有ペプチドが線維芽細胞の細胞外マトリクス生成に関与し、さらに表皮の水分低下、バリア機能低下を改善することでシワに対して効果があることが示唆された。

●キーワード | 食品機能成分、シミ、シワ、カシスアントシアニン、
コラーゲンペプチド

はじめに

現在、高齢化社会のさらなる加速が指摘され、皮膚科領域においても老化・加齢変化に関する研究が進められている。この皮膚の老化は、内因性老化と外因性老化に分

けられる。内因性老化とは生理的老化ともいい、各個人の遺伝子的素因を背景に生じる皮膚の加齢に伴う老化であり、形態的変化、機能的変化として表れる。一方、外因性老化とは内因性老化に環境因子、喫煙、紫外線照射による光老化などの環境要因による皮膚障害のダメージが蓄積して生じる

1882-3871/09/9-500\$05.00/0

Functional Food 2009 Vol.2 No.4 383

Exhibit A
page 2

発生 皮膚老化と脂溶性色素

老化である。外因性老化の光老化に關しては、日光に当たることの多い顔面などの露光部で顕著であり、シミ、シワなどの徴候として表れる。この皮膚の老化に伴って発生するシミやシワを改善・予防することは QOL (quality of life) 向上の観点からも重要であり、近年、食品機能成分を用いた改善・予防効果の研究が積極的に進められている。

1 シミの改善と予防効果

シミは皮膚基底層に存在するメラノサイトから産生される高分子色素メラニンが沈着し、発生したものである。メラニン産生の原因の一つとしては、紫外線照射が挙げられる。このメラニンは、メラノサイト内のメラノソームにおいてチロシナーゼが作用することで、チロシン、ドーパ [Dopa]、ドーパクロム (Dopachrome) を経て合成される¹⁾。このチロシナーゼ活性を阻害す

ることができればメラニン合成を抑制することができ、シミの改善・予防が可能となる。また、上述のメラニンの産生以外にも、顔面の血行不全によるくすみもシミの原因であると考えられている²⁾。

このシミを改善・予防する食品機能成分としては、ビタミンC、L-システイン、コウジ酸などの効果が知られている。コウジ酸に関しては、肝臓への影響の問題から2003年以降使用が中止されたものの、ビタミンC、L-システインを配合した食品やサプリメントは多く市販されている。最近、我々はカシスアントシアニンのシミに対する予防・改善を示唆する結果を得たので、以下、カシスアントシアニンのシミに対する作用について概説する。

カシスは欧州で消費量の多いベリー-果実であり、豊富に含まれるポリフェノールの一種であるD3G (アントシアニンはデルフィニジン-3-グルコシド)、D3R (デルフィニジン-3-ルチノシド)、C3G (シアニジン-3-グ

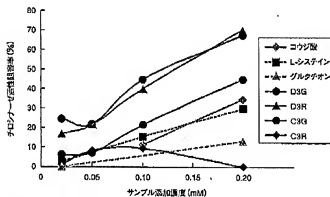


図1 カシスアントシアニンのチロシナーゼ活性阻害 (実験4より一部改定して示す)

チロシナーゼ、ドーパ配合液にD3G、D3R、C3G、C3R、別院としてコウジ酸、L-システイン、グルタチオンを0.025~0.2mM添加し、生成されるドーパクロム量を測定した。カシスアントシアニン系3種のドーパクロムを100として、その生成量と比較することでチロシナーゼ活性阻害率を算出した。

6. 食品機能成分のシモ、シワの改善と予防効果

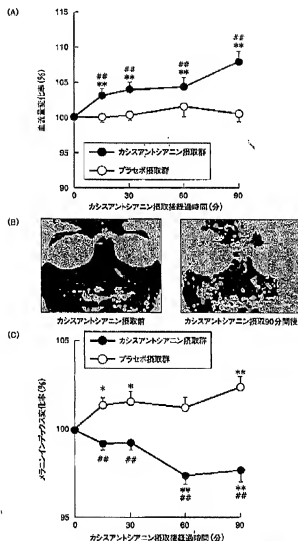


図27 カシスアントシアニン摂取による肌前血流量とメラニンデクスの変化

(A) カシスアントシアニン摂取後の血流量変化率、(B) 摂取5分より90分まで(90分)は原則10分間隔で撮影、(C) カシスアントシアニン摂取後のメラニンデクス変化率
 摂取前値との比較: * $p < 0.05$, ** $p < 0.01$, プラセボ群との比較: ** $p < 0.01$, 平均値±標準誤差

図3 カシスアントシアニンのシミ改善・予防効果概観

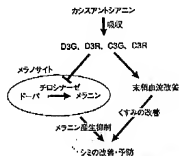


図3 カシスアントシアニンのシミ改善・予防効果概観

ルコシド), C3R (デルフィニジン3,4,5-トリノシド) で構成されている。アントシアニン は主に胃部と小腸上部から吸収され末梢組織を改善することが知られている³⁾。したがって、カシスアントシアニンを経口摂取することにより、末梢循環不良による顔色の赤みの低下などで生じるくすみに対して有効である可能性が考えられる。同時に、チロシナーゼ阻害活性が認められたため、カシスアントシアニンのシミに対する作用について検討した。

チロシナーゼを用い、D3G, D3R, C3G, C3R を各々0.025～0.2mM添加した際のドーパから生成されるドーパクオモヌをサンブル無添加時の生成量と比較評価した⁴⁾。D3G, D3R, C3G, C3R添加時のチロシナーゼ活性阻害率は、0.2mM D3Gは67.5%, 0.2mM D3Rは70.1%, 0.2mM C3Gは45%とコウジ酸やレスチニンより高いチロシナーゼ阻害活性を示した (図1)。

以上のことから、ヒトでシミを改善・予防する効果を検討した。30～45歳の健康女性被験者33名にカシスアントシアニン50mgを含む飲料100mLとカシスアントシアニンを含む飲料100mLとを単回摂取するクロスオーバー二重盲検試験を行い、

摂取後の顔の血流量変化をレーザードップラー血流計で、内眼角下部のメラニンインデックスをメタメーター-MQ38で測定し、新顔で比較した⁵⁾。その結果、カシスアントシアニン摂取群はプラセボ群と比較して摂取15分後より顔の血流量が有意に増加し、メラニンインデックスが有意に低い値を示した。この結果から、カシスアントシアニン摂取により、皮膚色が明るくなることが示唆された (図2)。しかし、このヒト試験は単回摂取試験であるため、今後長期摂取による検証が望まれる。

以上の結果をまとめると、摂取したカシスアントシアニンが吸収され、メラノサイトに作用し、チロシナーゼ活性を阻害することと血流改善作用によりくすみを改善することにより、シミの改善・予防に働くことが期待できる (図3)。

2 シミの改善・予防効果

シミは、生理的老化による乾酪と細胞の機能低下で起こるコラーゲン線維、弾性線維などの細胞外マトリクス量の低下によって組織が萎縮することで発生する。また、光老化においては紫外線に曝露されること

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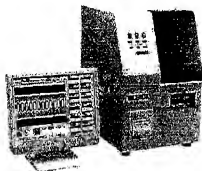
MCFAN Micro Circulant Array Flow Analyzer

装置外観

赤血球変形能、白血球活性度が一目で観察できます。
血液の流れを観察できます！

MCFANは毛細血管を模倣し、簡単な操作で血液の流れを直接顕微鏡観察・記録が出来る装置です。予防医学や健康食品、医薬関連の研究開発用として、お役立て下さい。

エムシーファン
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装置に関するお問合せは
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MCFAN Micro Channel Array Flow Analyzer

装置の特長

特長

1. 毛細血管を模倣したシリコンチップ流路にて、血液の流れを直接モニターで観察できます。



サンサラ状態

※事実上日本へモニタリング専用接続

2. 流路内血液が流れる通過時間を測定できます。
3. 流路を通過する細胞の形状状態をモニターで観察できます。



フローロ状態

使用例

1. 赤血球変形性の観察
2. 白血球活性化(粘着性)の観察
3. 血小板凝集性の観察

シリコンチップ

1. 流路幅4μm～7μmを標準チップとし、目的に合わせて選択できます。
2. 流路形状をカスタムデザインする事により、装置の応用範囲が広がります。

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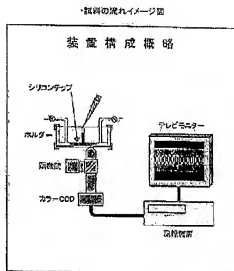
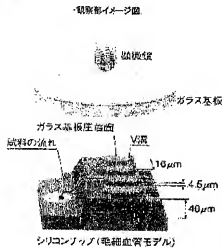
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MCFAN Micro Channel Array Flow Analyzer

測定原理



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**Vascular
Pharmacology**

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Possible mediators involved in decreasing peripheral vascular resistance with blackcurrant concentrate (BC) in hind-limb perfusion model of the rat

Kellian Iwawaki-Kamishige^{a,b}, Ramon V. Lavigne-Santos^a, Hiroshi Marumoto^b,
Takeshita Phrasang^a, Hiroshi Azuma^{a,*}

^a Department of Biophysics, Institute of Pharmacology and Experimental Pharmacology, Graduate School, Chiba Institute of Science, Chiba 260-8501, Japan

^b Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^c Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^d Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^e Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^f Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^g Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^h Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

ⁱ Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^j Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^k Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^l Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^m Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

ⁿ Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^o Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^p Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^q Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^r Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^s Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^t Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^u Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^v Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^w Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^x Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^y Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^z Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{aa} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ab} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ac} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ad} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ae} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{af} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ag} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ah} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ai} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{aj} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ak} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{al} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{am} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{an} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ao} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ap} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{aq} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ar} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{as} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{at} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{au} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{av} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{aw} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ax} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ay} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{az} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{ba} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{bb} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{bc} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

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^{be} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

^{bf} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

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^{bi} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

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^{bk} Department of Pharmacology, Institute of Medicine, Chiba University, Chiba 260-8501, Japan

Exhibit C
page 1

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3. Pharmacological analysis of the decrease in perfusion pressure with BC

As shown in Fig. 7, after the application of 100 mg/kg of XTC, the plasma concentration of XTC in the control group ($n = 3$) was significantly increased (12.1416 $\mu\text{g/L}$) as compared with the control group ($n = 3$) without affecting the extent of the XTC-induced convulsions. The convulsions were significantly attenuated in the XTC-treated group ($n = 3$) when the convulsions were induced by the XTC-treated group ($n = 3$) without affecting the extent of the convulsions. The convulsions were significantly attenuated in the XTC-treated group ($n = 3$) when the convulsions were induced by the XTC-treated group ($n = 3$) without affecting the extent of the convulsions.

• **What is the purpose of the study?**

Physiology 44 (2002) 211–221

pressure under the contraction caused by 60 mM KCl, at which the output was similar to the 10 μ M phenylephrine-induced one. The decrease in the perfusion pressure with 60 mM KCl under the contraction caused by 60 mM KCl was determined to be 50.9 \pm 2.2%, of the control decrease under the contraction with phenylephrine ($P < 0.05$, $n = 4$). The decrease in the output was also calculated by the ratio of the output at 60 mM KCl to that of phenylephrine (60 μ M) and was 50.9 \pm 2.2% as an inhibitor of Na⁺/K⁺ ATPase (McDonnell and Vane, 1973). So we can modify the decrease in perfusion pressure with 60 mM KCl to 50.9 \pm 2.2% of the control, $n = 5$ and 57.6 \pm 0.7% of the control, $n = 6$, respectively.

Results are shown in Fig. 7. The cyclic GMP levels in the perfusate remained unaffected before and after the addition of

to 10 μ M phalloidin; see above. The observed perturbation in the presence of 10 μ M phalloidin was not the result of a perturbation by 3C itself, because the transverse channel, by phalloidin binding, is not blocked. The perturbation was accompanied by the presence of 1 mM tubercydimine, which was accompanied by the significantly increased cyclic GMP levels in the perfused MC. Increased cyclic GMP from a basal level of 50.74 \pm 1.1 ng/ml to 9.8 \pm 0.8 \pm 1.1 ng/ml ($P < 0.05$, $n = 6$); at 1.1 ng/ml to 0.010 \pm 0.2 ng/ml ($P < 0.05$, $n = 7$); at 2.5 ng/ml. The increased cyclic GMP level with 1.8 and 2.5 ng/ml MC could easily be related to the basal levels in the presence of 30 μ M tubercydimine, which failed to modify the basal cyclic GMP concentrations, which failed to modify the basal cyclic GMP level.

3.4. Possible molecular(s) other than NO for the decrease in perfusion pressure with BC

We investigated the possible involvement of other than NO in

Fig. 4A, B. In the presence of 30 μ M nitroarginine, catalase and

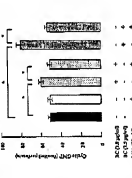
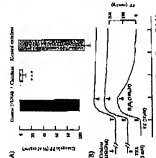


Fig. 3. Cyclic GMP levels in the perfusate under the several experimental conditions. All experiments were performed in the presence of 1 mM theophylline and 30 nM IBMX. Basal ($-BC$) and stimulated cyclic GMP levels with 1.8 and 2.5 μ g/ml BC ($+BC$) were compared ($n=3$). Cyclic GMP levels were significantly increased by BC ($P<0.05$). The increased cyclic GMP levels with BC were abolished in the presence of 30 nM cAMP antagonist 8-Br-cAMP.

E. Fournier-Karabegović et al. / *Vib.*[illegible]

concentration of 1250 U/ml as endogenous peroxidase. The decrease in peroxidase pressure with 50% and 75% of the control, $p < 0.005$, $p < 0.01$, 4A). Meanwhile, the inhibitory effect of cobalt disappeared after the enzyme solution is 60 °C for 30 min (2A,B). In the control, $n = 4$. In addition, exogenously applied H_2O_2 of 100 μ mol produced a sustained and potent increase in the peroxidase pressure during the consecutive measurement (Fig. 4B, 4C). The rates of $n = 4$ and by 10 add cobalt (Fig. 4B, 4C).

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$P=1.000$, $n=9$) was included. A total of 100 *in vitro* matured oocytes were fertilized with 100 spermatozoa and the resulting zygotes were cultured in combination with 100 *in vitro* matured oocytes (Lange et al. 2009).

Possible involvement of potassium channels in decreasing the perfusion pressure with BCC (LA 50'0'') was investigated as

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old reaction does afford the correct purification pattern with BGE (1.4:3:12.5% of the current for purification) (see Fig. 3).



Fig. 3. Decrease in the peak-on pressure (PP) with succinylcholine (A) and benzocaine (B) in the hand-tooth perfusion model of the rat. A bolus application of benzocaine (10 mg/kg) (A) and succinylcholine (2.5 mg/kg) (B) did not decrease but slightly increased the peak-on pressure during the contraction caused by 1.0 g/kg pharyngostimulant (PS).

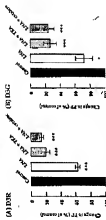


Fig. 7. Photomicrograph analyses of the dentures by peridone prepared with D18 (A) and D40 (B) in 3,4,4-trimethyl perfluoro methyl methacrylate. The decrease in the compressive pressure with D18 (A) or D40 (B) was significantly increased by 35 MPa above [DMA] above, i.e., add hexamethylenetetramine (HMTA) or 1,2,3,4-tetrahydro-2H-pyran (THP) at 40°C. $\times 1000$.

We tried to analyze the possible mechanism by decreasing the inhibition of the Ca^{2+} release by the presence of thapsigargin, by the addition of a calmodulin antagonist. The addition of thapsigargin (10 μM) to the perfusion pressure with Ca^{2+} Meantone, the inhibitory effect of calmodulin disappeared, but the Ca^{2+} release did not increase. The addition of thapsigargin (10 μM) to the perfusion pressure with Ca^{2+} Meantone, the inhibitory effect of calmodulin disappeared, but the Ca^{2+} release did not increase.

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It has been reported that oral intake of EC significantly decreases the peripheral circulation in human volunteers [Maravou et al., 2003, 2004; Maravou et al., 2005]. Endogenous H_2O_2 has been shown to be a stimulator to relax the smooth muscles of arteries and veins [Mammi et al., 2005].

[illegible]

Abstract

	N	D/E	D/S	C/A	C/D
Percent		33.682	16.682	13.915	14.2
B)					
					D/S
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第3回(平成16年度)B3 BioFuture Research Encouragement Prize 研究奨励金賞
 制奨分野: 専攻生(博士課程の修)

カシス (*Ribes nigrum* L.) 抽出物による末梢血管抵抗低下機序
 ○倉重恵子^{1,2)}, Renzo Loyaga³⁾, 松本 均²⁾, 徳永隆久²⁾, 東 洋¹⁾
 (東京医歯大・生材研・制御、¹⁾明治製菓・食料健康総合研、
²⁾東京医歯大・医・産婦人科)

【目的】「カシスポリフェノール(以下BCと記す)」は、カシス濃縮果汁を粉末状にした機能性食品素材である¹⁾。BCのヒトでの健康機能改善効果²⁾ならびに末梢血管改善効果^{3, 4)}が確認されているが、作用機序は不明である。BCにはdelphinidin-3-glucoside (D3G), delphinidin-3-rutinoside (D3R), cyanidin-3-glucoside (C3G), および cyanidin-3-rutinoside (C3R)の4種のアントシアニンが含まれているので、BCのラット後肢末梢抵抗血管拡張作用機序を詳細に解析するとともに、4種のアントシアニンの寄与についても併せて検討した。
 【方法と結果】ラット内臓骨動脈内に挿入したカニューレを介して収容クレス液を定流量循環し、遠流圧の変化を記録した。Phenylephrine (10⁻⁶M) 誘発収縮下にBCを添加すると遠流圧は徐々に低下した(Fig.1)。同作用は濃度依存性的で(Fig.2), eylele GMP産生増加を伴っており、血管内皮除去後には消失した。さらに同作用は、一酸化窒素合成酵素阻害剤(nitroarginine)と非特異的K⁺チャネル阻害剤(tetraethylammonium)との併用または nitroarginineとcatalaseとの併用により完全に阻害された。H₂O₂濃度によってBC作用に類似の遠流圧低下が観察され、catalaseならびにtetraethylammoniumはこれを抑制した。また、nitroarginine存在下、BC誘発遠流圧低下は、薬理学的性質の異なる特異的K⁺チャネル阻害剤(barium chloride, ibexlotoxin, 4-aminopyridine, charyldotoxin + apamin)によって部分的に抑制された。作用強度は異なるものの4種のアントシアニンは何れも遠流圧低下作用を示し、アントシアニン作用の総和はBC作用に匹敵した。また、主要アントシアニン、D3GならびにD3R作用はBCの場合と同様、nitroarginine + tetraethylammonium またはnitroarginine + catalaseにより阻止された。
 【結論】BCの末梢血管抵抗低下作用は血管内皮依存性でありNOおよび過分極因子(EDHF)産生/遊離の増加を介して観察されることが示唆された。さらに、H₂O₂がEDHFの有力候補物質と考えられ、種類の異なる種類のK⁺チャネルを活性化させる結果、過分極と末梢血管拡張をもたらし可能性が示唆された。BCの末梢血管抵抗低下作用において4種のアントシアニンが主要な役割を果たしている可能性が示唆された。

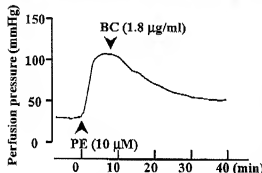


Figure 1. A sustained and progressive decrease in the perfusion pressure produced by blackcurrant concentrate (BC) in a concentration of 1.8 μg/ml during the contraction caused by 10 μM phenylephrine (PE).

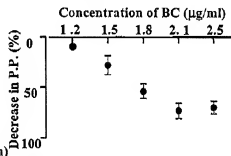


Figure 2. Concentration-dependent decrease in the perfusion pressure with blackcurrant concentrate (BC) in the hind limb perfusion model of the rat.

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Exhibit C
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